TEMPORAL AND SPATIAL VARIATION OF SHELL MICROSTRUCTURE OF *POLYMESODA CAROLINIANA* (BIVALVIA: HETERODONTA)

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ABSTRACT

Temporal and spatial variation of the microstructure of inner surface of shell, condition index and organic content of shell of the Carolina marsh clam *Polymesoda caroliniana* (Bosc) in three different Mississippi habitats are described and discussed in relation to one another and environmental conditions. Microstructure of the inner shell surface distal to the pallial line showed distinct seasonal variation but little spatial variation. Pseudospiral microstructure, on inner surface of shell undertucked by the periostracum, predominated over "normal" crossed-lamellar microstructures in cooler seasons. Presence and seasonal frequency of occurrence of complex crossed-lamella one inside the pallial line reflected habitat differences. It was consistently present in submerged clams, present only in June and September in wild clams, and absent in exposed clams. Survival and condition index of transplanted clams in submerged area were higher than those clams in areas often exposed to air. Condition index showed seasonal and spatial variation, while organic content did not.

The shell microstructure of a bivalve is determined by its genome. This genotype sets constraints that fix limits within which adaptive change can occur. Moreover, while basic molluscan shell microstructures are few (Taylor *et al.*, 1969, 1973; Gregoire, 1972; Carter, 1980; Watabe, 1981; Wilbur and Saleuddin, 1983; Carter and Clark, 1985), subtle variations within each structural category occur because details of shell crystallization can be influenced by environmental factors (Barker, 1964; Taylor *et al.*, 1969; Rhoads and Panella, 1970; Lutz and Rhoads, 1978, 1980; Carriker *et al.*, 1980; Carter, 1980; Prezant and Chalermwat, 1983; Lutz and Clark, 1984; Carter and Clark, 1985; Prezant and Tan Tiu, 1986; Tan Tiu, 1987; Tan Tiu and Prezant, 1987). Conservative shell microstructures can be important characters used to determine phylogeny (Carter, 1980). Furthermore, consistent, inducible microstructures could be used to monitor recent or past environmental conditions (Lutz and Rhoads, 1980). Thus, it is important to examine shell microstructural variations to reasonably evaluate the environmental significance of microstructural patterns. The goal of this study was to investigate the extent of shell microstructural variation, temporally and spatially, on the eurytopic Carolina marsh clam, *Polymesoda caroliniana* Bosc, 1801.

Aragonitic shells of Corbiculidae, like the marsh clam, consist of outer crossed-lamellar and inner complex crossed-lamellar layers, separated from each other by a distinct (or indistinct) myostracum (Taylor *et al.*, 1973). Shell microstructure of *Polymesoda caroliniana* has not been previously examined in detail except for the conchiolin layers within the shell (Kat, 1985). Taylor *et al.* (1973) briefly described the shell microstructure of a related species, *Polymesoda anomala* (Deshayes, 1855), from Ecuador.

MATERIALS AND METHODS

Specimens of *Polymesoda caroliniana*, ranging 11 to 43 mm maximum anterior posterior length, were collected seasonally (June, Sept, Dec 1985, Mar, June 1986) from a marsh at the Rod and Reel Fishing Camp, Old Fort Bayou, Jackson County, Mississippi, U.S.A. Each seasonal sample was treated similarly. Thirty specimens were shucked in the field. After shell length, height and width were measured, shells were preserved in absolute ethanol for later examination by scanning electron microscopy. Areas of inner shell surface examined and compared are shown in figure 1. Another fifty specimens were transported to the laboratory where

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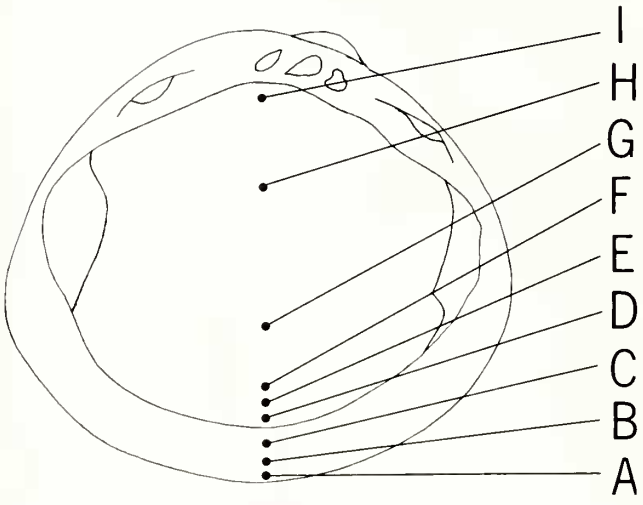

Fig. 1. Left valve of *Polymesoda caroliniana*. Areas of the shell surface examined are marked by dots, corresponding to the letters on the right (A, area undertucked by periostracum. B, area just dorsal to Area A. C, area between Area B and pallial line. D, E, F, the "transition zone". G, area at the level of ventral margin of adductor scars. H, area at the level of dorsal margin of adductor scars. I, area near umbo).

length, width, height, total weight with and without mantle water, shell and tissue dry weight, and organic content of shell were measured. Condition index and organic content of shell were computed. Definitions, procedures and care of specimens followed those by Prezant and Tan Tiu (1986) and Tan Tiu (1987).

A large sample of *Polymesoda caroliniana*, 11 - 43 mm long, collected in June 1985 from the Rod and Reel Fishing Camp, were marked and divided into two groups. One group was transplanted to a continually submerged area, and the other to a periodically exposed marsh area. Submerged and exposed areas are located within a 100 m radius of Halstead Bayou (adjacent to Gulf Coast Research Laboratory), Ocean Springs, Jackson County, Mississippi. Each group consisted of eight cages each containing 45 individuals. Details of procedures for marking, care of samples, size of cages and how they were set are similar to those described for studies of *Corbicula fluminea* Müller, 1774 by Tan Tiu (1987). Two cages were recovered from each site each season (beginning September 1985) at the same time wild samples were collected from the Rod and Reel Fishing Camp. Samples were treated as previously described. Because of high mortality, all cages in the exposed area were recovered in December 1985.

Monthly measurements of air, ground, surface and bottom water temperatures, water conductivity, dissolved oxygen, pH, methyl orange alkalinity, total filtrable residue, turbidity, transparency (Secchi depth), salinity, hardness, calcium, water depth and organic content of sediment were made in the three sampling areas. Water was absent in the emerged area on several occasions (June, July, Nov, and Dec 1985). Thus, water parameters could not be measured at those times. Details of methods used and errors of measurement are described in Tan Tiu (1987).

Significance of seasonal and habitat variation in condition indices and organic content of shell determined by one-way ANOVA, followed by Tukey test when ANOVA was significant. When only two samples were compared, t-test was used. Subjective evaluation was made in cases where statistical evaluation was not possible. All statistics were compared with critical values at $\alpha = 0.05$ and critical values used were conservative. Statistical methods used are described by Zar (1984).

Clams collected in the marsh at Rod and Reel Fishing Camp from June 1985 to June 1986 that were not in cages nor marked will be referred to as "wild" clams or group. Clams in cages transplanted to Halstead Bayou will be referred to as "experimental" groups. Experimental clams that were placed in the continually submerged area will be referred to as "submerged" clams or group, while clams that were placed in a regularly exposed area will be referred to as "exposed" clams or group.

RESULTS

ENVIRONMENT

The macroflora of the Rod and Reel Fishing Camp marsh (the location of seasonal wild samples and original source of experimental samples) and the exposed marsh area (a transplantation site), is predominantly *Juncus roemerianus* Scheele, while the submerged area (another transplantation site) was devoid of vegetation and had a muddy substratum. Turbidity, salinity, pH, calcium, total filtrable residue of water measured in the submerged area were significantly higher than at the Rod and Reel Fishing Camp (Table 1). During a few sampling periods (August to October 1985), when water was present in the exposed area, measurements of turbidity, conductivity, dissolved oxygen, methyl orange alkalinity and organic content of sediment were higher in the exposed area than in the submerged area at the same time. Temperature of water bottom, as measured by a maximum-minimum thermometer, ranged from 14.0 to 36.0°C in the submerged area and 7.9 to 42.2°C in the exposed area. Ground temperature measured at the bank of the submerged area ranged from 7.2 to 42.4°C. No maximum-minimum thermometer data are available in Rod and Reel Fishing Camp site.

SHELL MICROSTRUCTURE

Condescriptive statistics of the dimensions of shells examined by scanning electron microscopy are presented in Table 2. The inner shell surface of *Polymesoda caroliniana,* near the umbo (Area I), has irregular pits and grooves. Ventral to Area I (Areas G and H), the microstructures can be "clumped" into irregular mounds (Fig. 2), whose surficial borders represent areas where lamellae of opposing orientations meet (Fig. 3). The inner shell surface of areas G and H may be flattened with few mounds (Fig. 4), or underlain by irregular to granulate reticulated layers (Fig. 5).

The inner shell surface proximal to the pallial line (Areas D to I) can be divided into four microstructural types: complex crossed-lamella one, complex crossed-lamella two,

Table 1. Condescriptive statistics and t-tests of environmental variables measured at the Rod and Reel Fishing Camp and Submerged Area, Ocean Springs, Mississippi. Abbreviations in the variable column are as follows: surface water temperature (SWT), turbidity as measured by a nephelometer (Tur), water transparency as measured by a Secchi disc (Sd), conductivity (Con), dissolved oxygen (DO), salinity (Sal), methyl orange alkalinity (MOA), calcium (Ca), total filtrable residue (TFR), hardness (Hds) and sediment organic content (SOC). T-statistics of averages (with one standard deviation and n number of monthly measurements) are evaluated using critical values at α = 0.05 for (df) degrees of freedom. Min = minimum, max = maximum. Ho: Average values of environmental factors are the same in both places.

Variable	Rod and Reel Fishing Camp					Submerged Area					Significance		
	range		mean	standard deviation	n	range		mean	standard deviation	n	computed t	critical value	df
	min	max				min	max						
SWT (°C)	19.5	40.0	25.9	6.1	13	9.0	31.0	23.4	6.9	13	Student's t = 0.976	2.064	24
Tur (NTU)	4.4	20.0	8.0	4.4	12	5.0	26.7	12.9	6.8	12	Student's t = 2.118	2.074	22
Sd (cm)	30	93	66	23	12	35	65	46	11	11	Welch t = 2.823	2.120	16
Con (μmhos/cm)	100	12000	4898	4282	13	750	24000	8142	8901	13	Welch t = 1.184	2.110	17
DO (mg/L)	0	10.0	5.9	2.4	13	4.0	8.9	6.9	1.3	13	Welch t = 1.228	2.093	19
Sal (o/oo)	0	9.0	2.2	3.5	13	0	18.0	9.5	5.3	13	Student's t = 4.127	2.064	24
pH	6.2	7.6	6.7	0.6	12	6.6	8.5	7.4	0.6	12	Student's t = 2.689	2.074	22
MOA (mg $CaCO_3$/L)	5.0	77.5	30.8	21.8	13	4.0	22000.0	1738.4	6087.9	13	Welch t = 1.011	2.179	12
Ca (mg $CaCO_3$/L)	6.2	246.0	88.9	80.8	10	23.7	613.3	301.6	236.6	10	Welch t = 2.690	2.201	11
TFR (mg/L)	100.0	8100.0	3553.9	2749.8	13	433.0	21866.0	12377.0	7820.2	13	Welch t = 2.838	2.145	14
Hds (mg $CaCO_3$/L)	31.0	8200.0	1736.0	2969.7	8	92	13433.3	3124.0	4236.0	8	Student's t = 0.759	2.145	14
SOC (%)	11.6	29.06	24.09	6.06	12	6.51	14.90	9.85	2.17	12	Welch t = 7.667	2.160	13

complex crossed-lamella three and reticulate microstructure. Microstructure of the inner shell layer (Fig. 5) is always of the reticulate type.

Exposed tips of secondary lamellae in complex crossed-lamella one are variably shaped with broad surfaces, and are oriented almost parallel to the shell surface (Fig. 6). Exposed tips of secondary lamellae in complex crossed-lamella two are narrow and also variably shaped, oriented irregularly or obliquely to the shell surface (Fig. 7). Exposed tips of secondary lamellae in complex crossed-lamella three are also irregularly shaped, oriented almost perpendicular to the shell surface (Fig. 8) with lamellae extending farther out to the inner surface of shell than the neighboring lamellae. Reticulate shell microstructure consists of loosely to densely packed thin or thick meshwork that can be granulated (Fig. 9).

The microstructure of the inner shell surface in Area G can be grouped into irregular blocks (Figs. 10, 11). There is usually no detectable microstructure that represents a transition at the presumed "transition zone" (Areas D to F, just dorsal and adjacent to the pallial line) since this zone is frequently eroded. Thus, the dorsal boundary of the outer shell layer can be recognized as an elevated border or ridge along the curved antero-posterior axis. A convenient boundary between outer crossed-lamellar and inner complex crossed-

Table 2. Lengths of wild and caged *Polymesoda caroliniana* from Ocean Springs, Mississippi, whose internal shell surface microstructure was examined by scanning electron microscopy. Length measurements are in millimeter (min = minimum, max = maximum, S = submerged, E = emerged).

Date	Mean	Standard deviation	Range min	Range max	Total clams examined (n)
WILD					
June 1985	27.6	8.2	15.3	41.0	20
Sept 1985	29.3	4.9	20.0	37.6	29
Dec 1985	27.5	7.5	16.4	37.0	10
Mar 1986	33.4	4.5	24.5	38.5	10
June 1986	33.4	4.9	24.1	41.6	10
CAGED (S)					
Sept 1985	31.4	4.7	23.1	37.8	10
Dec 1985	34.1	3.3	27.3	41.5	30
Mar 1986	32.4	3.6	27.7	37.4	10
June 1986	34.7	5.2	29.2	41.5	10
CAGED (E)					
Sept 1985	30.8	3.7	25.2	36.0	10
Dec 1985	29.5	4.8	23.4	38.0	10

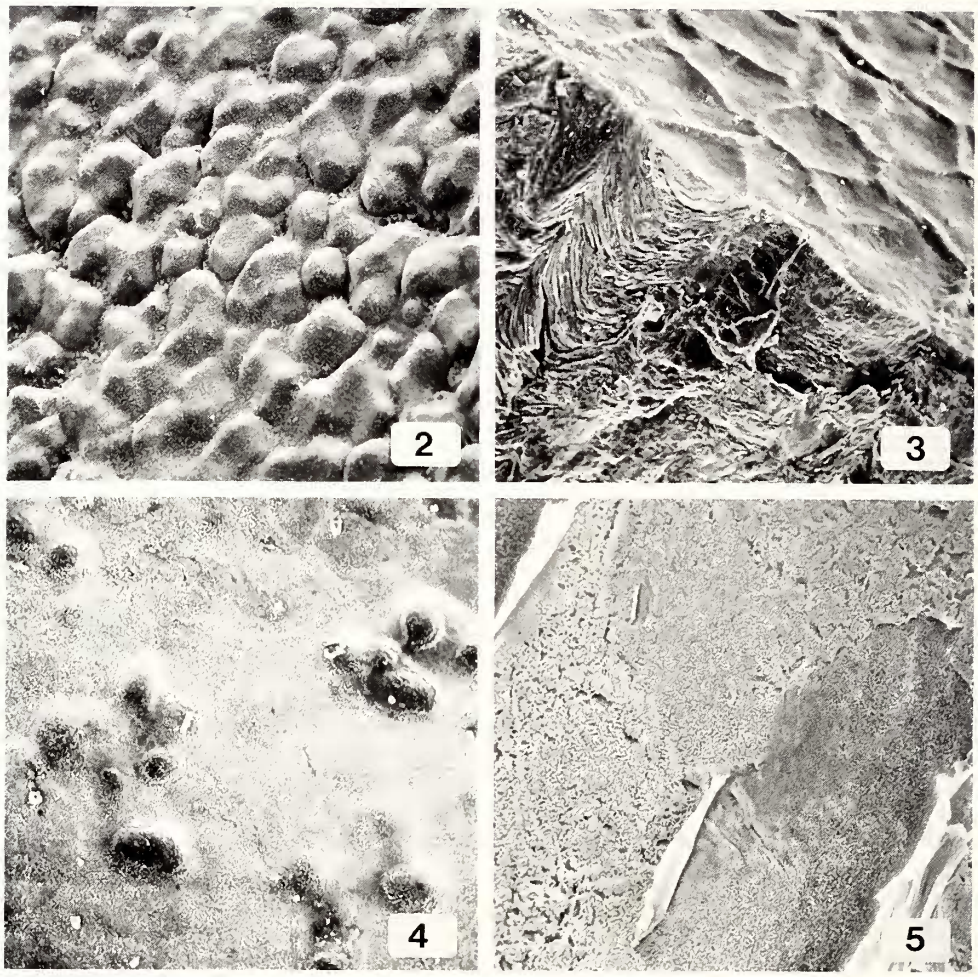

Fig. 2. Microstructures of inner surface of shell dorsal to the pallial line are grouped into irregular mounds. Mounds represent first order lamellae [Horizontal field width (HFW) = 352 μm. **Fig. 3.** Angular view of shell fracture dorsal to the pallial line. Second order lamellae of first order lamellae are oriented opposite to each other (HFW = 587 μm). **Fig. 4.** Few mounds are visible on rough surface of inner shell (HFW = 587 μm). **Fig. 5.** Irregular layers on surface of inner shell consists of reticulated microstructure (see Fig. 9) (HFW = 293 μm).

lamellar shell layers is therefore an eroded groove in place of an obvious pallial myostracum. This is evident at low magnifications (Fig. 12), where an apparent transition zone is seen only at a low magnification. Unlike the usually indistinct pallial myostracum, the adductor myostracum is distinct (Fig. 13). Both pallial (Fig. 14) and adductor (Fig. 15) myostraca can be traced sandwiched between the two shell layers.

Ventral to the myostracum (Areas A, B and C), the microstructures of the inner shell surface of *Polymesoda caroliniana* and *Corbicula fluminea* (Prezant and Tan Tiu, 1985, 1986; Tan Tiu, 1987) are similar, except that no spiral shell formations were observed in *P. caroliniana* during colder seasons. Adjacent and ventral to the myostracum, Area C, the exposed lath tips are irregularly arranged. Area C is often covered by an organic matrix that render the underlying structures indistinct. Laths in Area B, dorsal and adjacent to the area undertucked by the periostracum, are arranged regularly to form second order lamella. Direction of the second order lamellae are opposite to that of the adjacent first order lamella. Microstructure of the inner shell surface of both Area B and C are referred to as crossed-lamella two (Table 3). Reticulate microstructure with loosely arranged strands can be observed at times on Area B. The predominant microstructure of the inner shell surface in Area A (undertucked by periostracum) is crossed-lamella one in all three groups. Exposed tips of secondary lamellae in crossed-lamella one are irregularly arranged, neither forming rosette nor pseudospiral pattern. Microstructure C, a collective term of convenience referring to pseudospiral (Fig. 16) and rosette microstructure in *Polymesoda caroliniana*, is similar to that of microstructure C in *Corbicula fluminea*, except that in the former, no complete spiral was observed. In *P. caroliniana*, two arc-shaped secondary lamellae (Fig. 16) can be joined to one another to form an approximate circular structure. When overlain by organic matrix, the identity of each arc can be obscured, thus appearing as a continuous circular flat band. The tertiary lamellae, composing the hub of the arc secondary lamellae

are sometimes not aligned, such that the tertiary lamellar tips protrude at varying lengths into the central space of the circular structure. Therefore, the shape of the spaces enclosed by the secondary lamellae vary depending upon the degree of curvature of the secondary lamellae.

Seasonal and habitat variations in the microstructure of the inner shell surface of caged and uncaged *Polymesoda caroliniana* are summarized in Table 3. Over the 13 month period, microstructure C in wild clams was absent in June of 1985 and 1986, and its frequency of occurrence peaks in March 1986 (Table 3). The frequencies of occurrence of the following microstructures were highly correlated ($r >$ critical values at $r_{0.05(2)3} = 0.878$) in wild clams: microstructure C negatively correlated with crossed-lamella one and complex crossed-lamella one. Other microstructures of the inner shell surface did not show distinct seasonal patterns. Microstructure C was negatively correlated, whereas complex crossed-lamella one ($r = 0.941$) was positively correlated significantly with temperature of surface water at the time of sampling than the temperature average per season.

During the four seasons over the 12 month period, microstructure C in submerged clams was absent in September 1985 and June 1986, but its frequency of occurrence also peaks in March 1986 like that of wild clams (Table 3). Frequency of occurrence of crossed-lamella one was negatively correlated with that of microstructure C. Other microstructures of the inner shell surface did not show distinct

Table 3. Temporal and spatial variation of internal shell surface microstructure in *Polymesoda caroliniana*, Jackson County, Mississippi. Headings stand for areas of shell examined (first row) and shell microstructural type (second row). Shell microstructure abbreviations are: C, microstructure C; CL, crossed-lamella; CCL, complex crossed-lamella; Ret, reticulate. Frequency of occurrence expressed in percent, where 0 = 0%, 1 = 1 to 20%, 2 = 21 to 40%, 3 = 41 to 60%, 4 = 61 to 80%, 5 = 81 to 100%.

	Area A		Areas B-C		Areas G-I			
	C	CL1	CL2	Ret	CCL1	CCL2	CCL3	Ret
Rod and Reel Fishing Camp (wild)								
June 1985	0	5	5	0	1	0	5	0
Sept 1985	1	5	5	1	1	3	2	1
Dec 1985	2	3	5	0	0	3	3	1
Mar 1986	3	3	5	1	0	2	1	3
June 1986	0	5	5	0	1	1	1	3
Submerged Area (caged)								
Sept 1985	0	5	5	0	2	3	1	1
Dec 1985	1	4	5	1	1	2	2	2
Mar 1986	3	3	4	1	1	2	2	3
June 1986	0	5	5	0	1	1	1	4
Exposed Area (caged)								
Sept 1985	1	5	5	0	0	2	2	2
Dec 1985	3	3	4	1	0	3	0	3

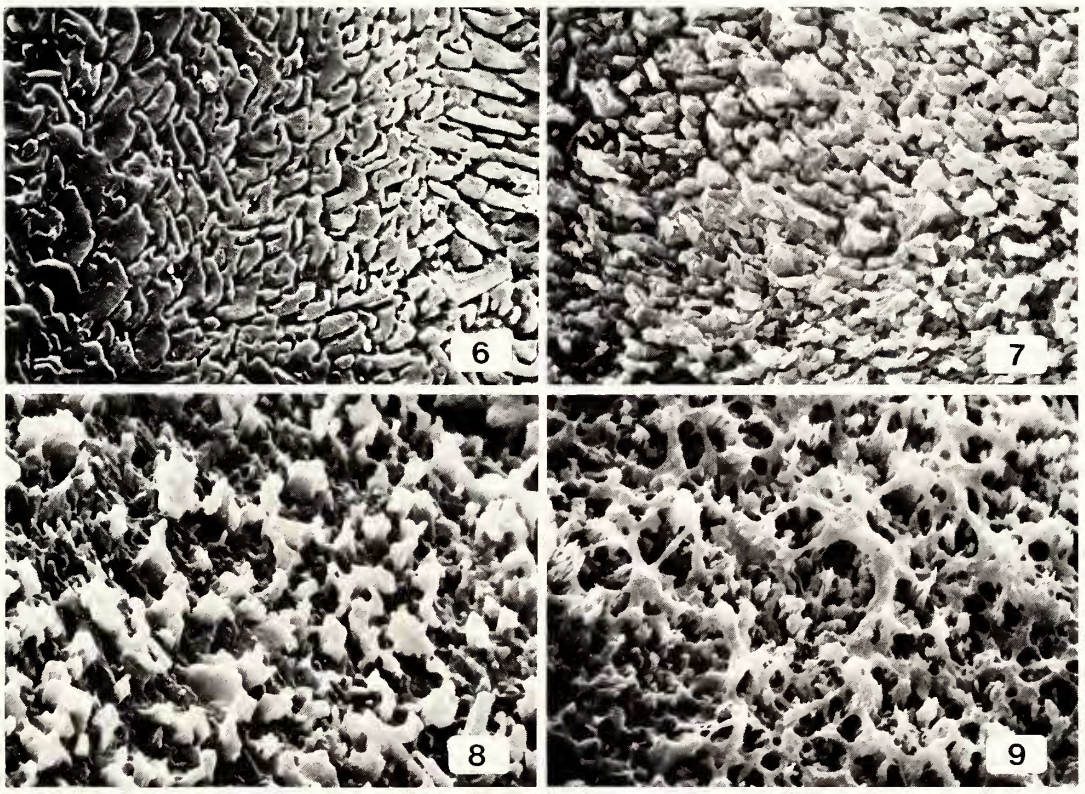

Fig. 6. Complex crossed-lamella one (HFW = 22 μm). **Fig. 7.** Complex crossed-lamella two (HFW = 22 μm). **Fig. 8.** Complex crossed-lamella three (HFW = 22 μm). **Fig. 9.** Reticulate microstructure (HFW = 22 μm).

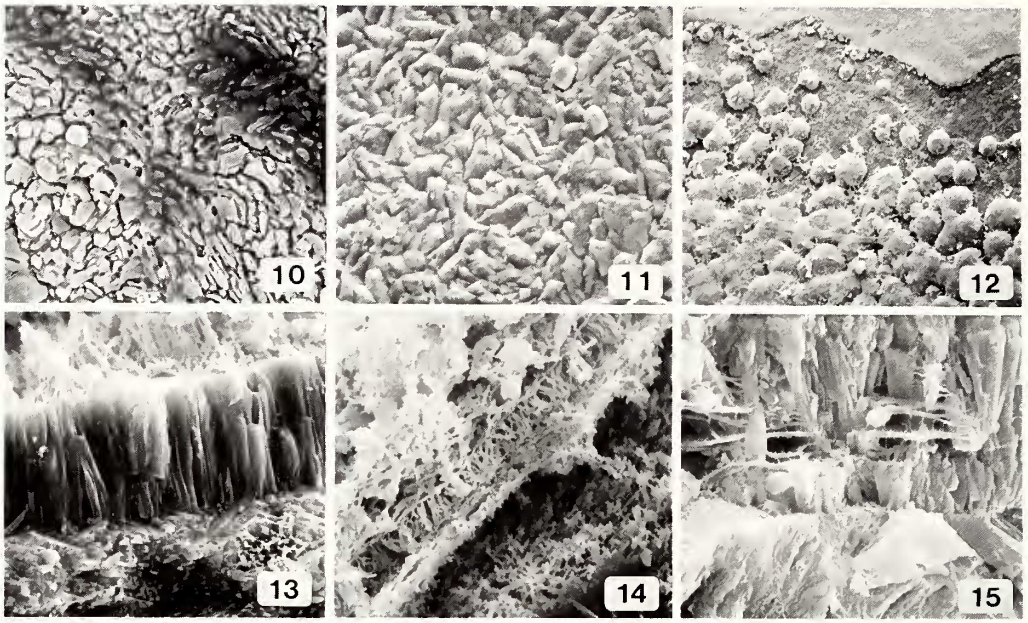

Fig. 10. Irregular blocks with smooth surfaces (HFW = 79 μm). **Fig. 11.** Irregular blocks with component secondary lamellae (HFW = 158 μm). **Fig. 12.** The groove is a convenient boundary between crossed-lamella (above) and complex crossed-lamella (below) (HFW = 790 μm). **Fig. 13.** Adductor myostracum consists of tall prisms. Remnant of adductor muscle at top of photo (HFW = 31 μm). **Fig. 14.** Organic compartments of pallial myostracum are sandwiched between naturally eroded shell layers (HFW = 16 μm). **Fig. 15.** Adductor myostracum is sandwiched between outer shell layer, crossed-lamella (above), and inner shell layer, complex crossed-lamella (below) (HFW = 32 μm).

seasonal pattern.

In exposed clams, available data on microstructure of the inner shell surface for September and December 1985 indicated that increase in the frequency of occurrence of microstructure C and decrease in crossed-lamella one were similar to those in wild clams. However, complex-crossed lamella one that was consistently present in submerged clams, present only in June and September in wild clams, were absent in exposed clams.

CONDITION INDEX AND ORGANIC CONTENT OF SHELL

Average percentages ($\pm$ one standard deviation, n) of condition indices in wild (June 1985 = 3.30 $\pm$ 0.97, n = 44, Sept 1985 = 2.64 $\pm$ 0.95, n = 45, Dec 1985 = 2.87 $\pm$ 0.96, n = 49, Mar 1986 = 4.00 $\pm$ 1.17, n = 50, June 1986 = 4.38 $\pm$ 0.99, n = 50), and submerged bivalves (Sept 1985 = 3.64 $\pm$ 1.53, n = 37, Dec 1985 = 4.60 $\pm$ 1.11, n = 11, Mar 1986 = 6.22 $\pm$ 1.06, n = 4) varied significantly as tested by ANOVA (Tables 4). In exposed clams, samples were available only for September 1985 (5.14 $\pm$ 0.80, n = 6). The Tukey test indicates that the condition index in wild clams can be divided into three groups; June-1985, September 1985, and March 1986-June 1986. Tukey test could not determine how the December condition index was related to either June or September 1985 groups (at least one type II error has been committed) (Table 4). Condition indices in submerged clams can be divided into September 1985 and March 1986 groups. A Tukey test could not determine how the December condition index was related to either September or March groups (Table 4).

Fig. 16. Arc-shaped secondary lamellae can join to form a hub in Area A (area undertucked by the periostracum) during cooler months (Dec. and Mar.) (HFW = 16 μm).

The condition index in December 1985 was significantly different among wild, submerged and exposed clams. Moreover, the Tukey test indicated that the condition index in wild clams was different from submerged and exposed clams (Table 5). A pairwise comparison of average condition index in wild and submerged clams also indicated that condition index in September (Welch $t = 3.522 > t_{0.05(2)7} = 2.365$) and March (Student's $t = 3.661 > t_{0.05(2)52} = 2.007$) were significant.

Analysis of variance of average percentages ($\pm$ one standard deviation, n) of organic content of shell did not show

Table 4. Temporal variation of means ($\bar{x}$) of condition index (CI) and shell organic content (SOC) in *Polymesoda caroliniana* from Rod and Reel Fishing Camp (wild) and submerged area (submerged), Ocean Springs, Jackson County, Mississippi.

						Analysis of Variance (one-way)						Tukey test
						Computed value			Table value			
	6/85	9/85	12/85	3/86	6/86	N	D	F	F	N	D	Overall conclusion
CI												
Wild	3.30	2.64	2.87	4.00	4.38	4	235	25.83*	2.85	4	200	$\bar{x}_1 \neq \bar{x}_2 \neq \bar{x}_4 = \bar{x}_5$
Submerged		3.64	4.60	6.22	—	2	49	6.95*	4.01	2	45	$\bar{x}_1 \neq \bar{x}_3$
SOC												
Wild	2.72	2.83	2.87	2.84	2.75	4	236	1.19	2.85	4	200	not necessary
Submerged		2.74	2.58	2.73	—	2	47	1.45	4.01	2	47	not necessary

*The ratio of the group mean square over the error mean square (F) with N and D degrees of freedom respectively is significant at $\alpha = 0.05$. When degrees of freedom fall between two table values, the lower value is used. CI and SOC are in %. Subscripts for $\bar{x}$ correspond to the order (left to right) of the means. Absence of available data is represented by blank spaces (before) and — (during) the sampling period.

Table 5. Tukey test among the average condition indices (CI) of *Polymesoda caroliniana* in three different habitats for December 1985. Computed studentized range (q) = ($\bar{X}_B - \bar{X}_A$) ÷ standard error. Critical value = 3.399 at $\alpha = 0.05$, degrees of freedom = 65 = 60, and total number of means tested = 3 (S = submerged, E = exposed area).

Habitat			Wild	Caged (S)	Caged (E)
Samples ranked by means (i)			3	2	1
Ranked sample means ($\bar{x}_i$)			2.87	4.60	5.14
Comparison (B vs A)	Difference ($\bar{X}_B - \bar{X}_A$)	Standard error	q	Conclusion	
1 vs 3	2.27	0.26	8.73	Reject Ho: $\bar{x}_1 = \bar{x}_3$	
1 vs 2	0.54	0.23	2.35	Accept Ho: $\bar{x}_1 = \bar{x}_2$	
2 vs 3	1.73	0.32	5.41	Reject Ho: $\bar{x}_2 = \bar{x}_3$	

Overall conclusion: $\bar{x}_1 = \bar{x}_2 \neq \bar{x}_3$

significant seasonal differences in wild (June 1985 = 2.72 ± 0.41, n = 48, Sept 1985 = 2.83 ± 0.38, n = 45, Dec 1985 = 2.87 ± 0.56, n = 49, Mar 1986 = 2.84 ± 0.40, n = 50, June 1986 = 2.75 ± 0.23, n = 49) (Table 4) and submerged clams (Sept 1985 = 2.74 ± 0.29, n = 35, Dec 1985 = 2.58 ± 0.16, n = 11, Mar 1986 = 2.73 ± 0.13, n = 4) (Table 4). In exposed clams, samples were available only for Sept 1985 (2.70 ± 0.19, n = 8). Average percentages of organic content of shell in Dec for wild, submerged and exposed clams were not significantly different as indicated by ANOVA ($F = 1.74 < F_{0.05(2)2,65} \approx F_{0.05(2)2,60} = 3.93$). Moreover, pairwise comparison of shell organic content in Sept (Student's $t = 1.167 < t_{0.05(2)78} = 1.991$) and Mar (Welch $t = 1.241 < 1_{0.05(2)9} = 2.262$) between wild and submerged clams was not significant.

DISCUSSION

Among the microstructures of the inner shell surface in Table 3, complex-crossed lamella one reflects habitat differences. Complex-crossed lamella one was present throughout the year in submerged clams, present only during June and September in wild clams, and absent in exposed clams. Environmental conditions in these three habitats were different. The submerged area was less stressful than the exposed area. Of 360 individuals in each group (exposed and submerged), 45% were recovered alive from the submerged while only 13% were recovered from the exposed area. "Stress can be said to occur when physiological (or other) processes are altered in such a way as to render the individual less fit for survival" (Bayne, 1980). Moreover, shell formation is costly. Shell formation involves ion transport, protein synthesis and sequences of physiological processes (Wilbur and Saleuddin, 1983). "Healthier" clams would therefore be expected to have more energy allocated for shell formation and maintenance than less "healthy" clams.

Among the internal shell surface microstructures observed in this study, microstructure C and crossed-lamella one were the most conservative in the sense that seasonal patterns (frequency of occurrence) among the three groups wild, exposed and submerged clams were almost similar despite differences in habitat. Frequency of occurrence of microstructure C is inversely associated with temperature of water surface at time of sampling in wild clams, and with average temperature of water surface in submerged clams. Difference in time response could be due to temperature stability provided by water to submerged clams in a continually submerged habitat.

Other than what has been discussed above, the seasonal and habitat variation nor the factor associated with the presence and frequency of occurrence of microstructure in the inner shell surface is not clear. Reticulate microstructure did not show seasonal variation instead increased in all three types throughout the experimental period. This microstruc-

ture is possibly a common response to altered environment induced by several factors.

Palmer (1983) reported that production of skeletal organic matrix can be more "demanding metabolically than the crystalization of calcium carbonate." Therefore, high amount of organic content of shell is expected to occur during the time when clams are "healthiest". However, organic content of shell did not show significant seasonal variation. Possibly the difference if any during the study period were diluted by the total content through the life of the animal as suggested by an anonymous reviewer of this paper.

In view of the data presented here and elsewhere (Tan Tiu, 1987; Prezant and Tan Tiu, 1986), it seems that microstructure of the inner shell surface outside the pallial line, especially on Area A (area undertucked by periostracum), although showing seasonal variation and slight habitat variation, is characteristic of some species. That is, while *Corbicula fluminea* can form spiral shell microstructures, *Polymesoda caroliniana* cannot. Shell outside the pallial line could indeed be a conservative characteristic of the species, and therefore could be used in taxonomic or phylogenetic analyses. On the other hand, shell microstructure beyond basic components inside the pallial line, by virtue of its greater variability (changes in shell ultrastructure due to formation, modification, dissolution, etc.) as a reflection of changes in shell physiology due to environmental changes, can be used for taxonomic purposes only if ontogeny and environmental history are known. The variability of shell ultrastructure outside the pallial line in other corbiculids needs further study.

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